

CLAIMS

WHAT IS CLAIMED IS:

1. A method for determining a nucleotide sequence of a nucleic acid by single dye molecule detection, which comprises the steps of:

- Sub B1
- 5 (a) immobilizing a nucleic acid molecule onto the surface of a solid;
- (b) annealing a primer, which has a sequence complementary to a part of the sequence of the nucleic acid molecule, to the nucleic acid molecule;
- (c) providing a solution which contains DNA polymerase and one type of dye-labeled dNTP (where N is A, T or U, G or C), or RNA polymerase and
- 10 one type of dye-labeled NTP (where N is A, U, G or C), to said immobilized nucleic acid molecule, and allowing the nucleotide to react with the 3' end of said primer, whereby a nucleotide, which forms a base-pair with a base opposed to the reaction site, is bound to the primer by action of the polymerase;
- 15 (d) detecting a bound, dye-labeled dNTP or NTP;
- (e) disrupting the dye molecule of the bound, dye-labeled dNTP or NTP;
- (f) repeating said steps (3) to (5) while changing the type of dye-labeled dNTP or NTP in turn, to sequentially bind dNTPs or NTPs which base-pair with the nucleotides of the nucleic acid molecule; and
- 20 (g) determining a nucleotide sequence of the nucleic acid molecule based on the types of the sequentially bound dNTPs or NTPs.

2. The method of Claim 1, wherein said surface of a solid is the inner wall of a capillary.

Sub B2

3. The method of Claim 1, wherein said step (4) comprises optically detecting the dye

25 molecule of said dye-labeled dNTP or NTP.

Sub B2

4. The method of Claim 1, wherein said step (4) comprises exciting dye molecules by irradiation of a laser beam and detecting the thus released fluorescent signal.

5. The method of Claim 1, wherein said detection is performed using a confocal fluorescence microscope system.

Sub B3

5 6. The method of Claim 1, wherein said disruption of dye molecules in said step (5) is performed by irradiation of a laser beam stronger than that in the step (4).

7. The method of Claim 1, wherein said dye is a fluorescent dye.

8. The method of Claim 1, wherein said dye-labeled dNTP is labeled with rhodamine, tetramethyl rhodamine (fluorescein), Rhodamine 6G, fluorescein isothiocyanate, or 4-

10 fluoro-7-nitro-benzofurazon (Texas red).

9. The method of Claim 1, wherein said dye-labeled NTP is labeled with rhodamine, tetramethyl rhodamine (fluorescein) Rhodamine 6G, fluorescein isothiocyanate, or 4-fluoro-7-nitro-benzofurazon (Texas red).

10. The method of Claim 1, wherein said dNTP and NTP is each labeled with the
15 same dye.

11. The method of Claim 1, wherein said solution consists of a droplet in which an aqueous solution containing said dye-labeled dNTP or NTP, is entrapped within a hydrophobic liquid.

12. A method for determining a nucleotide sequence of a nucleic acid by single dye
20 molecule detection, which comprises the steps of:

(a) immobilizing a primer, which has a sequence complementary to a part of the sequence of a nucleic acid molecule to be measured, onto the surface of a solid;

(b) annealing the nucleic acid molecule to the primer;

25 (c) providing a solution which contains DNA polymerase and one type of

- dye-labeled dNTP (where N is A, T or U, G or C), or RNA polymerase and one type of dye-labeled NTP (where N is A, U, G or C), to the immobilized nucleic acid molecule, and allowing the nucleotide to react with the 3' end of the primer, whereby a nucleotide, which forms a base-pair with a base
- 5 opposed to the reaction site, is bound to the primer by action of the polymerase;
- (d) detecting the presence of a bound, dye-labeled dNTP or NTP;
 - (e) disrupting the dye molecule of the bound, dye-labeled dNTP or NTP;
 - (f) repeating said steps (3) to (5) while changing the type of dye-labeled
 - 10 dNTP or NTP in turn, to sequentially bind dNTPs or NTPs complementary to the nucleotide of the nucleic acid molecule; and
 - (g) determining a nucleotide sequence of the nucleic acid molecule based on the types of the sequentially bound dNTPs or NTPs.
13. The method of Claim 12, wherein said surface of a solid is the inner wall of a
- 15 capillary.
14. The method of Claim 12, wherein said step (4) comprises optically detecting the dye molecule of said dye-labeled dNTP or NTP.
15. The method of Claim 14, wherein said step (4) comprises exciting dye molecules by irradiation of a laser beam and detecting the thus released fluorescent
- 20 signal.
16. The method of Claim 15 wherein said detection is performed using a confocal fluorescence microscope system.
17. The method of Claim 12, wherein said disruption of dye molecules in said step (5) is performed by irradiation of a laser beam stronger than that in the step (4).
- 25 18. The method of Claim 12, wherein said dye is a fluorescent dye.

19. The method of Claim 1, wherein said dye-labeled dNTP is labeled with rhodamine, tetramethyl rhodamine (fluorescein), Rhodamine 6G, fluorescein isothiocyanate, or 4-fluoro-7-nitro-benzofurazone (Texas red).
20. The method of Claim 1, wherein said dye-labeled NTP is labeled with rhodamine, 5 tetramethyl rhodamine (fluorescein) Rhodamine 6G, fluorescein isothiocyanate, or 4-fluoro-7-nitro-benzofurazone (Texas red).
21. The method of Claim 1, wherein said dNTP and NTP is each labeled with the same dye.
22. The method of Claim 9, wherein said solution consists of a droplet in which an 10 aqueous solution containing said dye-labeled dNTP or NTP, is entrapped within a hydrophobic liquid.
23. A kit, comprising:
- a) one or more solutions, each containing DNA polymerase and one type of dye-labeled dNTP, or
 - 15 b) one or more solutions, each containing RNA polymerase and one type of dye-labeled NTP, or both.
24. The kit of Claim 23, which comprises both a) and b).
25. The kit of Claim 23, which further comprises an immobilizing solid surface for nucleic acids.

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